

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraphs found on p.3:42 to p.4:18 as follows:

Generation of new catalytic activities in the novel method means that the enzymes having been subjected to the method are able to convert substrates which they were previously unable to convert, because the affinity of the enzyme for the substrate was too low (i.e., high K_M) and/or the rate of conversion (k_{cat}) too low (i.e., = high K_M) and/or the rate of conversion (= k_{cat}) of the enzymes was too low. In these cases, the ratio k_{cat}/K_M is zero or almost zero, i.e., catalysis does not occur. The generation of a new catalytic activity reduces the K_M or increases the k_{cat} , or both. A catalytic reaction occurs. The enzyme converts the new substrate after the mutagenesis.

It is possible in principle for new catalytic activities to be generated in all enzymes, and preferably new catalytic activities are generated in hydrolases in the novel method. Hydrolases form the 3rd class of enzyme (i.e., 3.-.-) (~~= 3.-~~) in the IUB nomenclature system. Hydrolases are preferred in the novel method because, as a rule, a simple detection reaction for them exists and, in many cases, they are used in industrial syntheses. It is particularly preferred to generate new enzymatic activities in hydrolases selected from the group consisting of proteases, lipases, phospholipases, esterases, phosphatases, amidases, nitrilases, ether hydrolases, peroxidases and glycosidases, very particularly preferably lipases, esterases, nitrilases or phytases.

Please amend the paragraph on p.5:31 to 6:2 as follows:

Particularly suitable for the methods according to the invention for producing mutations (step b, Figure 1) is the Escherichia coli strain XL1 Red (Epicurian coli XL-1 Red) ~~Escherichia coli strain XL1 Red (= Epicurian coli XL-1 Red)~~, which is marketed by Stratagene La Jolla, CA. It carries the following genetic markers: Δ [mcrA]183, Δ [mcrCB-hsdSMR-mrr]173, endA1, supE44, thi-1, gyrA96, relA1, mutS, mutT, mutD5, lac. MutS is a mutation in the mismatch repair pathway, mutT is a mutation in the oxo-dGTP repair pathway and mutD5 is a mutation in the 3'-5' exonuclease subunit of DNA polymerase III. Competent cells of this strain can be purchased from Stratagene under order number 200129. A functional derivative of this strain preferably means Escherichia coli strains which contain the following genetic markers: relA1, mutS, mutT and mutD5. These genetic markers result in a distinctly increased mutation rate in the organisms. They should therefore not be incubated on agar plates or in a culture medium for too long, because otherwise they lose their vitality.

Please amend the paragraph on p.6:4-33 as follows:

For detection of the newly generated catalytic activity, it is possible and advantageous, in the case where vectors have been used, for the DNA initially to be isolated from the E. coli strain XL1 Red or its functional derivative and be inserted into a microorganism which has no corresponding enzyme activity (step c, Figure 1). If, for example, an esterase is introduced into these selection organisms, these microorganisms must not have any esterase activity which cleaves the ester used for selection for the newly generated catalytic activity in the esterase. Other esterase activities in this organism do

not interfere with the selection. The introduction of the DNA can take place, as described above, using phages or viruses, by conjugation or by transformation. In the case of introduction via phages or viruses or by conjugation, specific isolation of the DNA is unnecessary. The DNA can be introduced directly, by conjugation or via the phage or virus, into the microorganism used for the selection. Thus, the transfer of the DNA takes place in these cases without isolation of the DNA, and in the case where vectors are used by a transformation. It is also possible for the DNA of the microorganisms which show the newly generated catalytic activity after selection to be introduced without isolation by conjugation or using phages or viruses or by transformation into the strain *E. coli* XL1 Red or a functional derivative for a further selection cycle (see Figure 1, dotted line) (~~= see Figure 1, dotted line~~). It is possible in this way for the method according to the invention to be performed one or more times in sequence. The DNA is in this case transferred from the *E. coli* strain XL1 Red or its functional derivatives to the selection organisms and finally returns to the *E. coli* strains for a new selection cycle.

Please amend the paragraphs on p.9:7-45 to read as follows:

The culturing conditions are established so that the organisms (i.e., *Escherichia coli* strain XL1 Red and selection organisms) (~~i.e., = *Escherichia coli* strain XL1 Red and selection organisms~~) grow optimally and that the best possible yields are obtained. Culturing is preferably carried out at from 15°C to 40°C, particularly advantageously from 25°C to 37°C. The pH is preferably kept in the range from 3 to 9, particularly

advantageously from 5 to 8. In general, the incubation time of from 1 to 240 hours, preferably from 5 to 170 hours, particularly preferably from 10 to 120 hours, is sufficient, but longer incubation times may also be necessary in a few cases for the mutagenesis or detection.

Please amend the table on page 10 as follows:

Enzyme from	Manufacturer
Pseudomonas cepacia (Lipase PS " <u>Amano</u> " [®])	Amano, Nagoya, Japan
Pseudomonas cepacia (Lipase AH " <u>Amano</u> " [®])	Amano, Nagoya, Japan
Acylase " <u>Amano</u> " [®] (ACS)	Amano, Nagoya, Japan
Rhizopus delamar (Lipase D " <u>Amano</u> " [®])	Amano, Nagoya, Japan
Rhizopus javanicus (Lipase F-AP15 [®])	Amano, Nagoya, Japan
Candida rugosa (Lipase AY " <u>Amano</u> " [®])	Amano, Nagoya, Japan
Mucor javanicus (Lipase M " <u>Amano</u> " [®])	Amano, Nagoya, Japan
Penicillium roquefortii (Lipase R " <u>Amano</u> " [®])	Amano, Nagoya, Japan
Penicillium cyclopium (<u>Lipase G</u> " <u>Amano</u> " [®] 50 Lipase G50 [®])	Amano, Nagoya, Japan
Chromobacterium viscosum (crude)	Toyo Jozo, Tokyo, Japan
Chromobacterium viscosum (pure)	Toyo Jozo, Tokyo, Japan
Rhizomucor miehei	Biocatalysts Ltd. Pontypridd, UK
Humicola lanuginosa	Biocatalysts Ltd. Pontypridd, UK
Rhizomucor miehei (Lipozyme [®] , immobilized)	Novo, Bagsvaerd, Denmark

Candida antarctica lipase B (<u>Novozym[®] SP 435</u> Novozyme SP435[®] , immobilized)	Novo, Bagsvaerd, Denmark
Candida antarctica lipase B (<u>Novozym[®] SP 525</u> Novozyme SP525[®] , free)	Novo, Bagsvaerd, Denmark
Candida antarctica lipase A (<u>Novozym[®] SP 526</u> Novozyme SP-526[®] , free)	Novo, Bagsvaerd, Denmark
Candida antarctica lipases A, B (<u>Novozym[®] SP 382</u> Novozyme SP-382[®] , free)	Novo, Bagsvaerd, Denmark
Pig liver esterase	Fluka, Buchs, Switzerland
Esterase from Thermoanaerobium brockii	Fluka, Buchs, Switzerland

Please amend the paragraph on p.11:7-8 as follows:

The esterase was mutagenized using the strain Escherichia coli XL-1 Red (Epicurian coli XL-1 Red) ~~Escherichia coli XL-1 Red (=Epicurian coli XL-1 Red)~~.